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SUPERCRITICAL FLUID EXTRACTION OF PCBs IN TANDEM WITH HIGH RESOLUTION GAS CHROMATOGRAPHY IN ENVIRONMENTAL ANALYSIS

by

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MANAGEMENT PERSPECTIVE

This paper was written in response to a request by the Organizing Committee of the 10th International Symposium on Capillary Chromatography for NWRI to make a presentation on the subject of supercritical fluid extraction on-line with gas chromatography. Our expertise in this field has been widely recognized all over the world.

NWRI supercritical fluid extraction and supercritical fluid chromatography research in environmental analysis covers most pertinent martices - sediments, fish tissue and water. The paper focusses on the practical extraction of PCBs from sediment samples using dynamic and static extraction. Extraction time is reduced at least 20-fold per sample (to less than 15 minutes) and provides additional safety protection to the analyst.

Dr. J. Lawrence
Director
Research and Applications Branch

PERSPECTIVES DE GESTION

La présente communication a été préparée en réponse au comité organisateur du 10e colloque international sur la chromatographie capillaire, qui demandait à l'INRE de présenter l'extraction sous pressions hypercritiques utilisée en série avec la chromatographie gazeuse. Notre expertise dans ce domaine a été largement reconnue dans le monde entier.

À l'INRE, les recherches en techniques d'extraction et en chromatographie sous pressions hypercritiques pour l'analyse environnementale couvrent la plupart des milieux visés - sédiments, tissus de poissons et eau. La communication porte surtout sur l'extraction technique des BPC à partir d'échantillons de sédiments grâce à des méthodes dynamiques et statiques. Le temps d'extraction est réduit au moins vingt fois pour chaque échantillon (à moins de 15 minutes) et assure une protection supplémentaire à l'analyste.

J. Lawrence, Ph.D.
Directeur
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ABSTRACT

A simple method has been developed for the direct coupling of supercritical fluid extractions with a high resolution gas chromatograph equipped with an electron capture detector. SFE conditions have been investigated for PCBs from sediments in terms of mobile phase, entrainer, pressure, temperature and mass-flow optimization. The dynamic and static extractions were compared. Extraction efficiency of up to 100% in 10 minutes has been obtained. The extracted PCBs are quantitatively transferred onto a fused silica open tubular column. Determination of PCBs was tested on certified sediment samples from NWRI and the results agreed well with certified values (S.D. < 3.5%).

RÉSUMÉ

Une méthode simple a été mise au point pour le couplage direct de l'extraction sous pressions hypercritiques avec la chromatographie gazeuse haute résolution et détection par capture d'électrons. Ce type d'extraction a été expérimenté pour les BPC des sédiments, et spécialement pour la phase mobile, le véhicule, la pression, la température et l'optimisation du débit massique. On a comparé les extractions dynamiques et statiques. Il a été possible d'obtenir un rendement d'extraction de 100 % en 10 minutes. Les BPC extraits sont transférés quantitativement dans une colonne tubulaire ouverte en silice fondue. Le dosage des BPC a été vérifié à l'aide d'échantillons de sédiments certifiés de l'INRE; les résultats obtenus sont en bonne corrélation avec les valeurs certifiées (écart-type < 3,5 %).

SUPERCRITICAL FLUID EXTRACTION OF PCBs IN TANDEM WITH HIGH RESOLUTION GAS CHROMATOGRAPHY IN ENVIRONMENTAL ANALYSIS

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Key Words :

Supercritical fluid extraction (SFE)
Polychlorinated biphenyls
Tandem SFE-HRGC-ECD

Summary

A simple method has been developed for the direct coupling of supercritical fluid extractions (SFE) with a high resolution gas chromatograph (HRGC) equipped with an electron capture detector (ECD). SFE conditions have been investigated for polychlorinated biphenyls (PCBs) from sediments in terms of mobile phase, entrainer, pressure, temperature and mass-flow through the extractor. The dynamic leaching and static steady-state extractions were compared. Extraction efficiencies of up to 100 percent in less than 15 minutes have been obtained. The extracted PCBs are quantitatively transferred into a fused silica open tubular column (OTC). Determination of PCBs was tested on certified sediment from National Water Research Institute and the results agreed well with certified values.

1 Introduction

There is great concern among environmental analytical chemists for monitoring the levels of organic contaminants in sediment, biota and fauna. The contaminants of interest, occur at low concentration and a great amount of time is required using standard analytical methodology for their extraction and cleanup. Greater sensitivity and reliable recoveries can be achieved through the use of larger samples combined with pre-concentration step directly onto a pre-column, or an easily accessible

adsorbent. Such preconcentration processes as adsorption on resins (1) and solvent extraction have been used after supercritical fluid extraction (SFE) with varying degrees of success. Trapping into a suitable solvent (2) and partitioning onto a stationary phase have also been suggested and seem to be attractive methods that should be further investigated.

Recently, Hawthorne *et al.*(3) described coupled SFE with gas chromatography using a flame ionization detector (FID) that yields quantitative recovery of polycyclic aromatic hydrocarbons (PAHs) at low ug/kg levels. Tandem SFE-HRGC greatly reduces the time required for extraction and concentration steps, and allows maximum sensitivity by quantitatively transferring the extract into the open tubular column (OTC), where it is partitioned in a stationary phase prior to GC analysis.

This contribution covers the application of both non-steady state and steady-state apparatus coupled directly to a HRGC system for PCB analysis. Advantages of the steady-state system will be discussed.

2 Experimental

2.1 Instrumentation

A Varian Vista 6000 gas chromatograph and Jones 7910 Series column heater with a Rheodyne 7125 injection valve were used to accommodate a heat exchanger for pre-heating the mobile fluid and the extraction cartridge above their critical temperatures.

The SFE system consists of a pump (P), an extractor (E) connected to a preheater (not shown) and a switching valve (V-1); these parts are located in the Jones 7910 Series column heater. The

outlet from the valve is connected to a 10 cm section of 25 to 30 um I.D. fused silica restrictor and an accumulator. Our

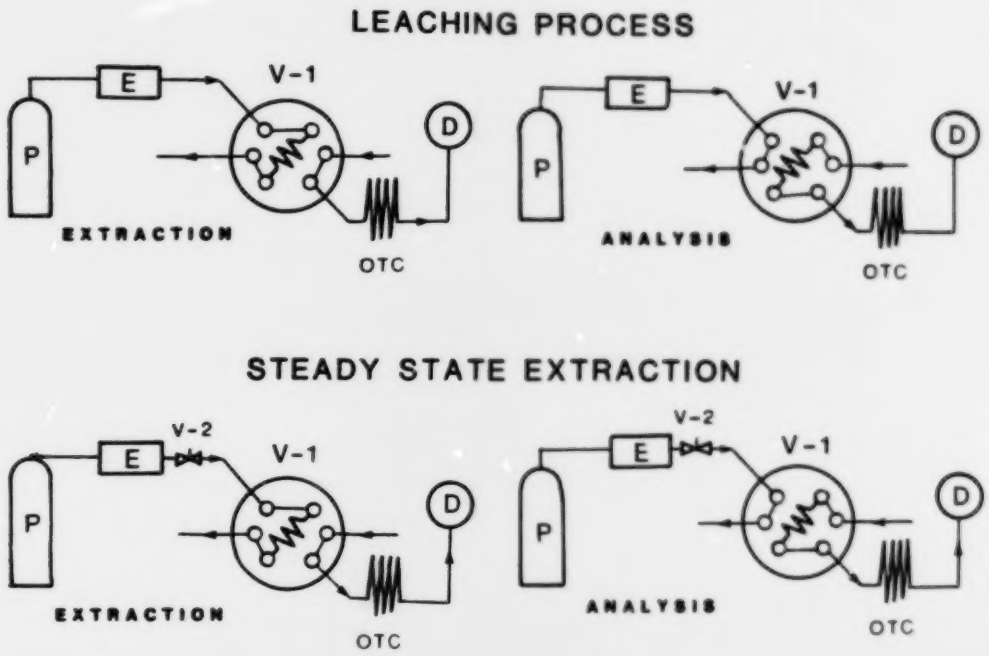

Figure 1. Schematics of the dynamic and static extraction system.

concept for coupling a supercritical fluid extractor to a gas chromatograph is shown in Figure 1 for a dynamic, non-steady state extraction system. The steady state, static, extraction system contains an additional on-off valve, which is located between the extractor and the accumulator. The connection between restrictor and the accumulator is achieved via a microreducing union (Gasukuro Kogyo, Tokyo, Japan, Cat.No.FI 11871) located inside the GC-oven. A brief description of individual parts follows :

2.1.1 Piston Pump

Our system consists of the ISCO uLC-500 pumping system. It can deliver a flow rate up to 30 mL/min of a fluid isobarically or in a pressure/density programming mode of operation.

2.1.2 Pressure Transducer

Indicates pressure in the extractor and its range is calibrated up to 10,000 psi (70 MPa).

2.1.3 Switching Valve

A Rheodyne Model 7125 6-port valve, installed in the column heater after the extractor outlet accommodates the restrictor and an accumulator. This valve is an important part of the system, forming an interface between the extraction unit and HRGC oven.

2.1.4 The extractor is the principal part of the system and is made from a machined stainless steel cartridge with connectors at either end for attachment to 1/16" O.D. stainless steel tubing. Samples are retained between two stainless steel frits of 2 um porosity. The internal volume of the extractor may vary between 0.5 to 100 mL but in general, its volume is approximately 2 mL. During the extraction step, the extraction cartridge is placed in a separate pre-heated column heater set to a desired extraction temperature. The column heater is a single compact unit requiring minimal bench space, and can be installed in a number of orientations from horizontal to vertical. Virtually any extraction cartridges and pre-column configurations up to 30 cm in length and up to 13 mm diameter may be employed. A pre-heater and extractor are located in a modular stacking aluminum block and the valve fits into a heated valve compartment equipped with easy access to the both ends of the extraction cartridge. A max. temperature setting of the extractor's oven is 199 °C.

2.1.5 The Restrictor regulates pressure in the extraction cartridge and releases it into an accumulator. The internal diameter of the outlet restrictor controls the rate at which eluent flows out of the extraction cartridge and therefore controls the volume of supercritical fluid used per extraction. For supercritical CO_2+2% CH_3OH flow rates through 10 cm long 25 um I.D. restrictor is 180 mL/min (S.T.P.) corresponding to 0.354 g/min, and 141 mL/min (S.T.P.) or 0.278 g/min for 20 um I.D. restrictor at 200 atm in agreement with ref. (4). Larger diameter restrictors permit higher extraction efficiencies per unit of time because a larger volume of supercritical CO_2+ 2% CH_3OH passes through the extraction bed.

2.1.6 Accumulator

The sensitivity attainable by this extraction system is made possible by incorporating a short "retention gap" pre-column (2m x 0.32mm I.D.; d_f= 1.2um) fused silica tubing coated with SE-54 and cross-linked. The analytes emerging from the extractor are partitioned in the pre-column, which is kept at sub-ambient temperature (5 °C) after depressurization through a restrictor. Desorption from the accumulator is achieved with hydrogen as a carrier gas. A rapid temperature ramp of the accumulator in the GC-oven to 140 °C removes the volatile analytes and transfers them quantitatively onto an analytical OTC. The simplest interfacing method is direct coupling of the restrictor to the accumulator through a switching valve or a standard cool on-column injector.

2.2 Interface

Tandem coupling of the SFE with a HRGC is made using a 6-port switching valve with connections to a restrictor and an

accumulator. The accumulator can be be connected to the OTC by switching the valve to another position. This arrangement requires minimum modification to the commercially available gas chromatographic equipment. It can be performed via a micro-union with zero dead volume graphite filled vespel ferrule or by direct insertion of the restrictor through a standard cooled on-column injector, which can be removed when required. Extracted analytes are partitioned inside the accumulator, which is located in the GC-oven equipped with CO_2 sub-ambient cooling accessory.

2.3 Operation

There are two techniques that can be applied to extract analytes from sediment samples. The operation in both modes are now briefly described.

2.3.1 Non-steady state, dynamic leaching procedure

The sediment sample is weighed and then transferred into the extractor. The extractor is installed in the pre-heated oven and the piston pump is programmed to reach required pressure as soon as possible but within 3 minutes. Valve 2 located below the extractor should be closed. However, when the required pressure is reached, the valve will be opened and leaching process runs according to the previously determined time interval. In our case, the leaching time for PCBs from the particular sediment at 200 atm and 40 °C is 10 minutes.

2.3.2 Steady-state, static extraction provides extracts under conditions reaching equilibrium in the extraction cartridge between concentrations of analytes in the extraction fluid and the concentration of the analyte in sediment. Instrumentation required does not differ significantly for operating in either

mode. It requires the installation of a second valve to the system. The most suitable place for installing the valve is between extractor and valve 1. This valve is usually closed for a required period to reach equilibrium between the S-L-V phases (S-solid; L-liquid; and V-gaseous phase). Two minutes is an adequate time for equilibration with no more than 30 seconds purging and re-loading the extractor with fresh supercritical fluid; this process is repeated 5 times in order to achieve a minimum 95 percent recovery from the sample. Following extraction the switching valve is adjusted to allow carrier gas to flow through the pre-column, accumulator and the analytical column thereby transporting the analytes from the accumulator to the analytical column for chromatographic separation and quantification.
Temperature programming is initiated after 1 minute from switching the valve 1 in position extraction to position analyze.

2.4 Setting Extraction Time

The time required for complete extraction of a particular matrix (sediment, fish tissue, water, etc) can be determined with relatively good precision. This extraction process can be monitored from a known spike using the same amount of a sediment by HRGC or by means of radiolabelled standards (4).

2.5 Sample Description

The sediment sample (EC-1), which is a certified standard, was obtained from the National Water Research Institute, Quality Assurance Section. The total PCB concentration in the sample was determined to be 2002 +22 ug/kg using both Soxhlet and sonification extraction techniques. Aroclor standards were

available from our depository and were prepared in isooctane and their concentration was reconfirmed by HRCG-ECD (5,6).

Table 1. EC-1 Sediment Reference Material PCB Content in mg/kg.

Method	Mean ($\bar{X}$) + S.D.
Ultrasonic	2.02 $\pm$ 0.07
Soxhlet	1.97 $\pm$ 0.08

Critical properties were calculated from general formulae of $C_{12}H_6Cl_4$ representing Aroclor 1242, $C_{12}H_5Cl_5$ for Aroclor 1254 and $C_{12}H_4Cl_6$ for Aroclor 1260 using the method of Ambrose (7) and corresponding data for vapor pressures from ref. (8).

Table 2. Calculated Values of Chemical and Physical Properties for Some Aroclors.

Parameter	Aroclor 1242	Aroclor 1254	Aroclor 1260
Molecular Weight	292.0	326.5	360.0
Crititical Temperature, K	816.7	909.0	955.6
Critical Pressure, atm	22.7	22.9	23.0
Boiling Point, K	578.0	652.0	683.0
Acentic Factor		0.479	
Triple Point, K		283.0	
Critical Volume, $cm^3/g.mol$		192.0	

The enhancement of solubility in supercritical carbon dioxide provides the basis for supercritical fluid extraction processes, and it is approximately 1000 for 80 atm and 40 °C; 10,000 for 100 atm and 100,000 for 130 atm as related to the steady state extraction.

2.6 Gas Chromatography

A Varian Vista 6000 gas chromatograph equipped with a SE-52 coated OTC (30m x 0.25mm i.d.) was used to separate components and an electron capture detector (ECD) was employed for their quantification. The average linear velocity for hydrogen as a carrier gas was 45 cm/s. An initial temperature of 5 °C was held during extraction. Afterward, it was programmed to 140 °C at 20 °C/min, followed by 2 °C/min to 240 °C. The detector makeup gas was nitrogen at a flow rate of 20 mL/min; its temperature was 350 °C. When supercritical CO_2+2% CH_3OH was used, we incorporated a T-piece between the accumulator and the analytical OTC to vent out CO_2+CH_3OH vapor. In this case, 50 cm x 0.53 mm I.D. fused silica tubing and a shut-off valve served very well. The peak areas were summed and converted to calibration units with the concentration response factors (5).

3 Results and Discussion

The effects of pressure, temperature and amounts of sediment samples required for the analysis using both static and dynamic methods were evaluated experimentally.

In the beginning, the leaching experiments were conducted to evaluate a matrix effect. Extractions of muffled and spiked sediment samples and periodic analyses of the extraction effluent from these samples using carbon dioxide were conducted at 10.35 MPa (100 atm), and 60 °C. The complete recovery of PCBs were achieved at 10.35 MPa and 60 °C within 8 minutes. Results of four 100 mg replicate samples (averaged value) are shown in Figure 2.

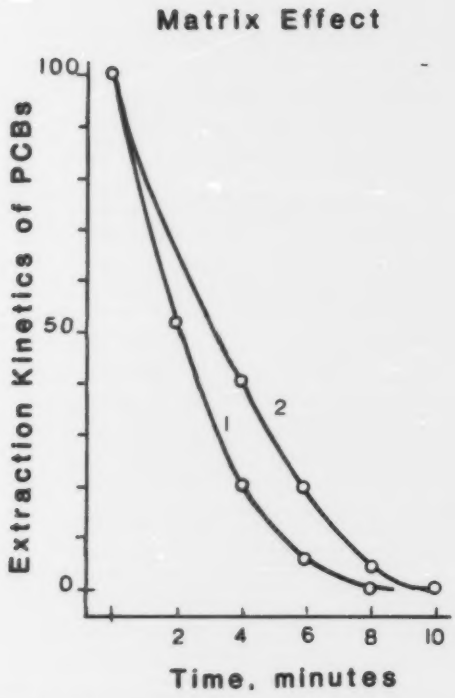

Figure 2. Kinetics of leaching PCBs with carbon dioxide from muffled sediment (1) and certified sediment (2) at 10.35 MPa and 60 °C; n=4.

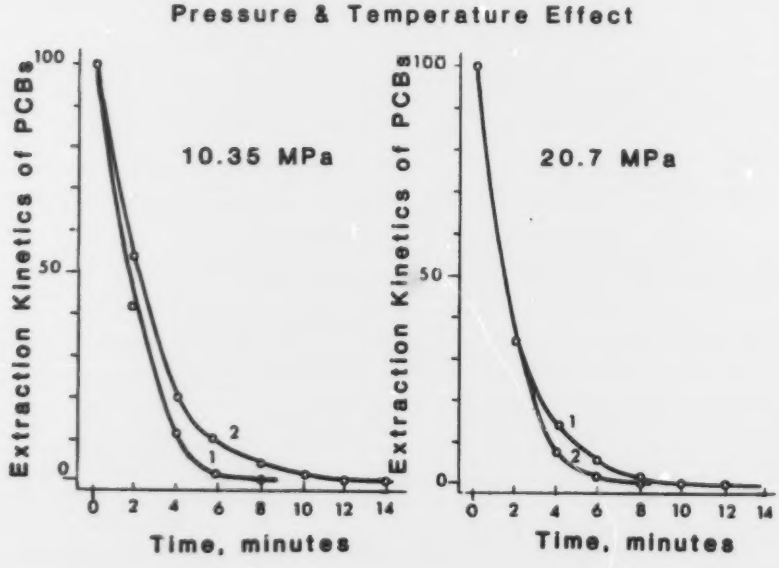

Figure 3. Kinetics of leaching PCBs with carbon dioxide from certified sediment EC-1 at 10.35 MPa, 40 °C (1) and 60 °C; (2) and 20.7 MPa at two temperatures (n=4).

Similar experiments conducted on the certified standard sediment, EC-1 showed a negligible effect of the muffling process. The carbon dioxide extraction of four parallel-averaged data is shown in Fig.3. It is evident from these results that solubility at lower pressures and lower temperatures is sufficient for extracting the PCBs with 100 percent yield within 10 minutes.

Effects of pressure and temperature were also investigated. In the dynamic mode of leaching the PCBs from sediment matrices, increasing the pressure (and in this case the mass flow) through the extractor apparently increased the extraction efficiency of PCBs. At higher pressures higher flow-rates pass through the extractor. As shown in Fig. 3,extraction of PCBs from sediment at the same pressure (10.35 MPa) and two different temperatures takes longer time at higher temperatures due to the viscosity increase. The effect was minimal when two different pressures at the same temperature was compared. Another advantage of lower pressure is amount of co-extractants that show a significant increase at higher pressures and affect quantitation process.

Even though carbon dioxide is a suitable mobile phase for extracting PCBs, we decided to investigate the addition of a small amount of methanol as an entrainer. Using an entrainer concentration of 2 percent, it was found that a reversed effect is observed. The solubility of PCBs in these mixed solvents is greater than in carbon dioxide, thus increased pressure affects the extraction efficiency significantly, as it is demonstrated in Fig.4. In essence, higher pressure shows greater extraction efficiencies but there is no substantial evidence that simultaneous increase of temperature and pressure plays an important role. It was observed that extraction time decreased at

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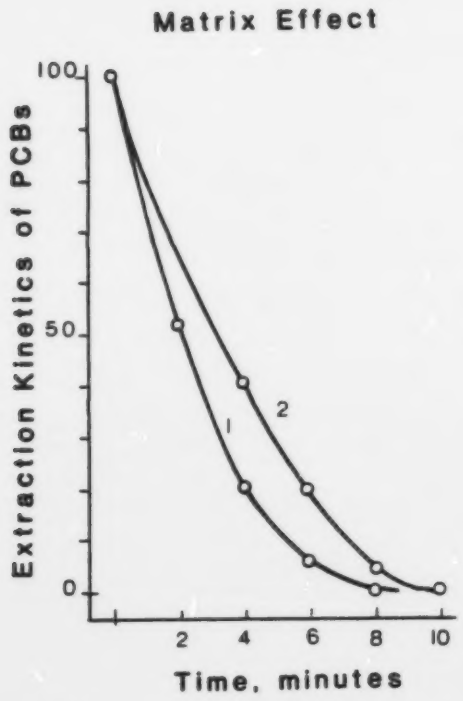

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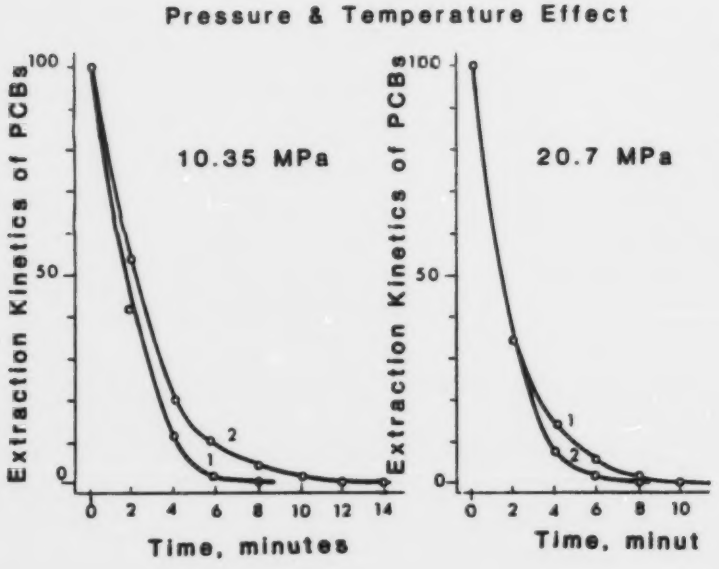

Figure 3. Kinetics of leaching PCBs with carbon dioxide from certified sediment EC-1 at 10.35 MPa, 40 °C (1) and 60 °C; (2) and 20.7 MPa at two temperatures (n=4).

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higher pressure of cca 20 percent.

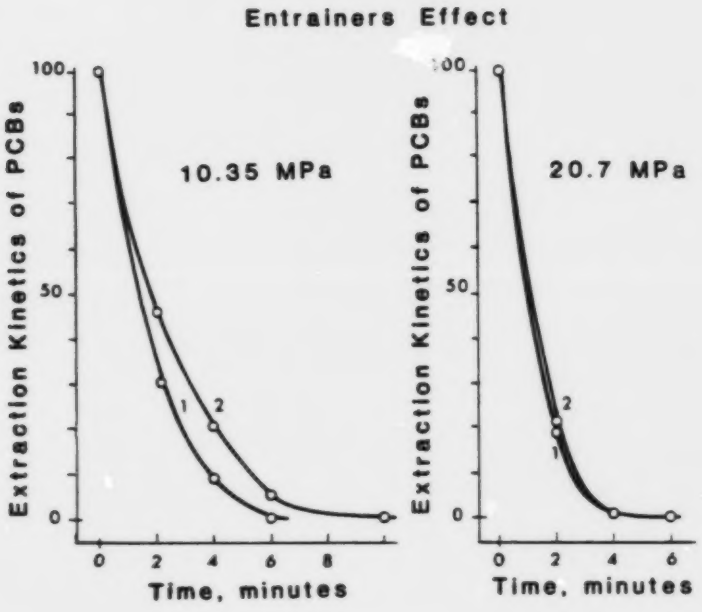

Figure 4. Kinetics of leaching PCBs with carbon dioxide + 2% CH_3OH from certified sediment at 10.35 MPa and 20.7 MPa at 40 °C (1) and 60 °C (2); n=4.

Based on these findings, our static extraction procedure was optimized by selecting carbon dioxide with 2 percent methanol as the mobile phase (extractant), and setting extraction conditions to optimize the required pressure to 20.7 MPa at 40 °C.

Results showing kinetics of extraction efficiencies are presented in Fig. 5. Just to emphasize the matrix effect, it is evident that SFE procedure must consider it as a significant parameter in order to obtain quantitative recoveries. As it was shown in our previous study, the moisture content also affects the extraction kinetics (9). However, this parameter was not evaluated.

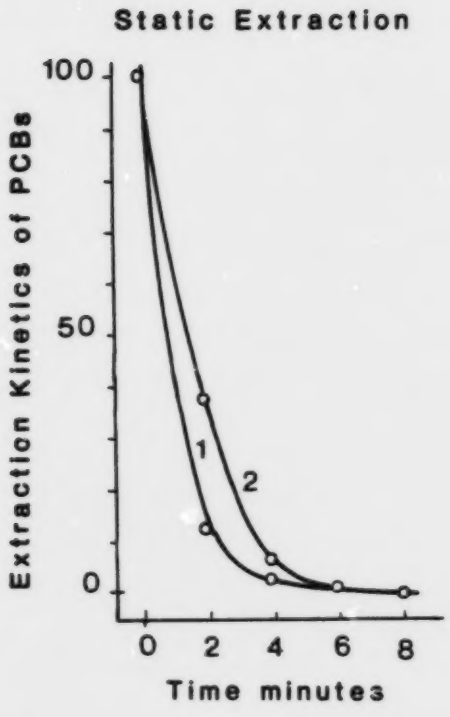

Figure 5. Kinetics of static extraction with carbon dioxide + 2% CH_3OH for muffled and certified EC-1 sediments at optimized conditions of 20.7 MPa and 40 °C; n=4.

Tandem SFE-HRGC-ECD provides a new approach to the extraction and analysis of organic pollutants from environmental matrices and has several potential advantages over traditional extraction methods. Since the extracted pollutants are quantitatively transferred into an OTC, maximum sensitivity is achieved, thus reducing the quantity of sample required and minimizing safety hazards associated with classical sample preparation methodologies.

A comparison of SFE-HRGC-ECD analyses for four different quantities of the certified PCB standard sample were repetitively run 6 times using the static extraction method. The equilibrium time was set to 2 minutes with 30 sec transfer into the pre-

column. This procedure was repeated 5 times. The pre-column was held at 5 °C and expanded mobile phase in gaseous state was vented out, thus the OTC was not contaminated with methanol. Afterwards, the carrier gas (hydrogen) was introduced into the pre-column and temperature of the GC-oven was increased to 140 °C. At this moment the shut-off valve on the T-piece connector was closed and the analysis began. Results are tabulated in Table 3, and represent the mean of 6 replicates.

Table 3. Static Extraction of the Certified EC-1 Sediment using Carbon Dioxide + 2% Methanol at 20.7
Carbon Dioxide + 2% Methanol at 20.7 MPa and 40 °C.

Amount of Sediment (mg)	Certified PCB Amount (ng)	$\bar{X}$ (ng)	S.D.
10	20.2	20.90	1.885
30	60.6	60.05	3.130
60	121.2	120.68	3.370
100	202.0	201.47	2.610

It should be possible to analyze PCBs to much lower levels, if the impurities present in carbon dioxide could be diminished. In this case the static extraction significantly eliminates spurious peaks from the mobile phase, since its total amount passing through the extractor is smaller than when dynamic technique is used. The results in Table 3 demonstrate that the tandem SFE-HRGC-ECD technique is capable of providing quantitative data with an improved precision at a fraction of the analysis time per sample, eliminating a tedious cleanup and concentration step.

4 Conclusions

Tandem supercritical fluid extraction-gas chromatography with electron capture detection provides the potential for combined sample preparation and PCB-analysis to be accomplished within 50 minutes. In addition to automated operation, rapid analyses and high sensitivities with ECD can be achieved using steady state, static extraction methodology. The present work demonstrates its qualitative and for the first time also quantitative operation for PCB sediment extraction. Optimization of the extraction and separation processes is also discussed.

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